IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Adrian K West, *et al.*
App. No	:	10/517,653
Filed	:	March 8, 2005
For	:	METALLOTHIONEIN BASED NEURONAL THERAPEUTIC AND THERAPEUTIC METHODS
Examiner	:	Daniel E. Kolker
Art Unit	:	1649
Conf #	:	5626

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Adrian Keith West, a named inventor of the above-captioned application, declare and state as follows:

1. I received my Ph.D. degree in molecular biology from the University of Melbourne in 1987.

2. Currently, I am a senior member of staff at the Menzies Research Institute at the University of Tasmania. I have been at this position and related positions since 1987. I have been Associate Head, School of Medicine, University of Tasmania since 2007.

3. I am a co-inventor of the subject matter claimed in the above-captioned application. I have read and understand the specification of United States Patent Application No. 10/517,653. Additionally, I am familiar with the prosecution history of this patent application, including the subject matter of the currently pending claims.

4. I understand from the outstanding Office Action dated December 9, 2009 that the Examiner considered the Declaration filed on August, 20, 2009 as insufficient to

overcome the rejection of some of the pending claims based upon anticipation. More particularly, the Examiner asserted that the data presented in the previous Declaration would not support the conclusion that MT-2 enters the kidney but not the brain, especially due to two very different time points (i.e. 40 minutes vs four days). Applicants chose a time-point midway in the period mentioned by Penkowa and Giralt, which is 40 minutes, to monitor metallothionein (MT) presence in the brain tissue. The results showed that administration of MT, even in a 50 fold higher dose than as originally stated in Giralt and Penkowa, cannot be detected in the vicinity of a cryolesion under the conditions described by the references. Therefore, Applicants concluded that the methods taught by Giralt and Penkowa cannot deliver a sufficient amount of MT required to promote neuronal regeneration to a target tissue as claimed in the instant invention. However, the Examiner asserted that an equally parsimonious explanation would be that MT-2 takes somewhere between forty minutes and four days to enter tissue peripherally administration. With this view, the Examiner appears to assume that in Penkowa with the injection performed for three days neurite outgrowth would necessarily occur, in other words, a sufficient amount of MT required to promote neuronal regeneration to a target tissue would necessarily be delivered. *See* page 4, lines 10-29 of the Office Action.

5. Applicants are providing the following information to experimentally prove that Penkowa and Giralt method would be unable to deliver MT in a sufficient amount required to promote neuronal regeneration:

Scientific Rational

To determine whether MT administered therapeutically via intraperitoneal injection (i.e. administration method according to Penkowa and Giralt) is capable of reaching bioactive levels in the injured brain following cryolesion injury.

Methods

A. Six adult (5 month old) male MT knockout mice (genetic deletion of MT-1 and MT-2 genes) received a freeze injury (cryolesion) to the cortex region of the brain.

B. Immediately post-injury (within 3 minutes of injury), mice received an intraperitoneal (i.p.) injection of MT (175 μg rabbit Zn-MT2A in 100μl sterile saline)

C. Animals received further i.p. injections of MT (same concentration) for the next three days (four injections in total)

D. Three animals were harvest at four days after the cryolesion, and three animals at seven days after the cryolesion was performed.

E. One MT knockout mouse was left unwounded and uninjected (as an experimental control)

F. Protein samples were collected from brain, and analyzed by western blotting to determine the presence of MT.

Results

Applicants did not observe any protein bands (white lines) in the 7kDa size range (the size of MT) in any of the animals that received MT injections, in brain. *See* Figure 1, which is presented as Exhibit A. This indicates that MT was not detectable in the brain tissue. Based upon calibration western blots (*See* Figure 2, which is presented as Exhibit B), the minimum level of MT protein detection we can achieve is ~10ng MT. In this experiment, Applicants loaded 3.3μg total protein into each lane of the western blot gel, and observed no MT signal, meaning that there was less than ~3μg MT per mg of protein in the tissue samples.

The protein bands (white lines) in Figure 1 (Exhibit A) that appear in the gel with brain tissue above 49kD in size are non-specific protein bands that are experimental artifacts. This is because the same bands appear in all lanes, including Lane 1 (control animal that received no MT injection). These bands are most likely endogenous immunoglobulins that non-specifically react to the antibodies that are used to detect MT.

Conclusion

Applicants did not observe MT in the brain after it was therapeutically administered via intraperitoneal injection into mice that had received a cryolesion injury to the brain, at either four or seven days postinjury. Based upon the detection limit of this technique, we are confident that MT had not accumulated in the brain to levels reaching ~3μg MT per mg of brain protein. In contrast, as disclosed in the Examples 1-2 of the specification, the claimed method delivers a substantial amount (e.g. 1 to 10 μg/ml for the specific test condition of the

Examples) of metallothionein to the injured rat cortex and stimulates neurite formation, elongation and outgrowth. Hence, we have shown that intraperitoneal injection of MT according to Penkowa and Giralt is exceedingly unlikely to reach bioactive levels in the brain.

In the foregoing experiment, Applicants have administered MT in accordance (same number of injections but 10-fold greater amount of MT than Penkowa or 5-fold greater than Giralt) with the study of Penkowa and Giralt, who suggested (but did not show) that they observed MT in the brain after cryolesion injury to adult mice. Hence, it is unreasonable to believe that the beneficial outcomes of MT that Penkowa and Giralt observed following therapeutic administration of MT following cortical cryolesion were due to MT reaching the brain in bioactive concentrations.

Hence, it is our contention from this data that the observations of Penkowa and Giralt that i.p. injection of MT following cryoinjury to the brain leads to brain recovery (but not specifically neuron regeneration – they never looked) could not have occurred by MT reaching the brain at bioactive concentrations.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 6 May 2010

Adrian Keith West, Ph.D.

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Exhibit A

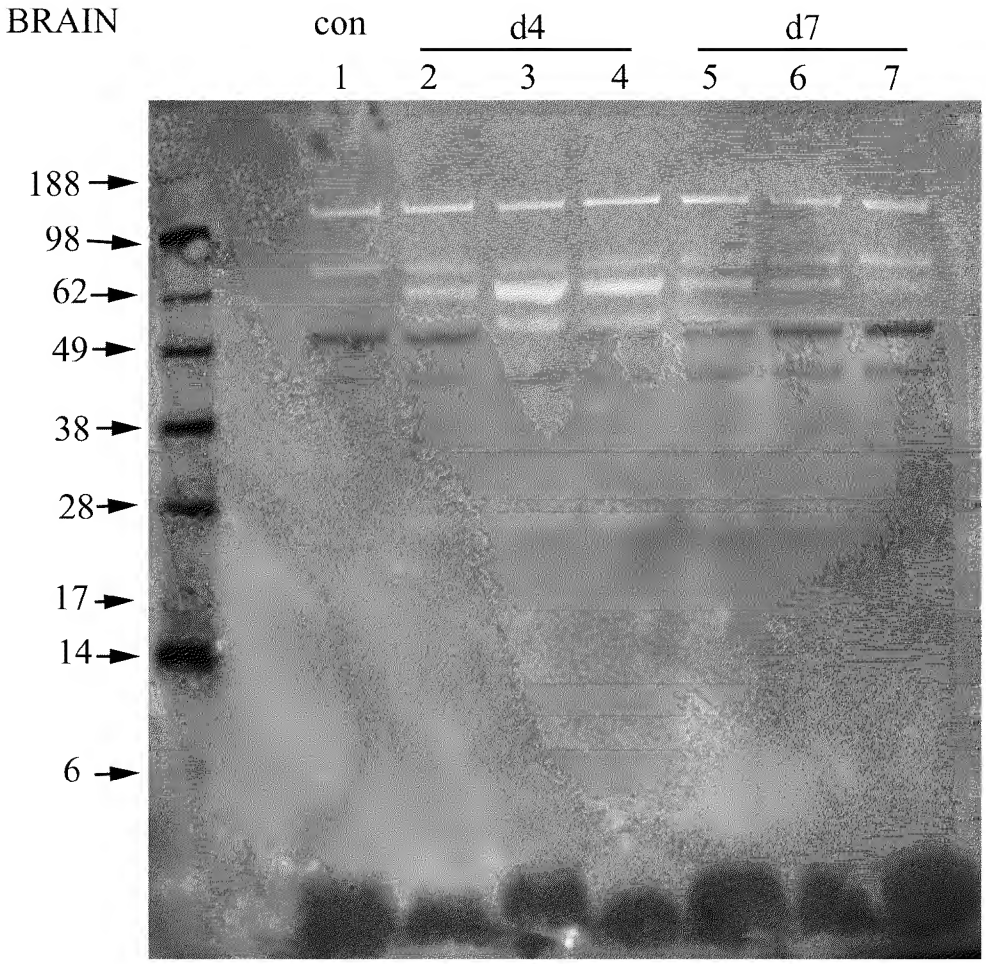

Figure 1 Western blot. Transgenic mouse brain cryolesions were treated with metallothionein administration by intraperitoneal route. Control sample (track1), samples harvested at four days after cryolesion (d4; tracks 2-4), and samples harvested at seven days after cryolesion (d7; tracks 5-7) are shown.

Exhibit B

Standard curve of MT concentrations

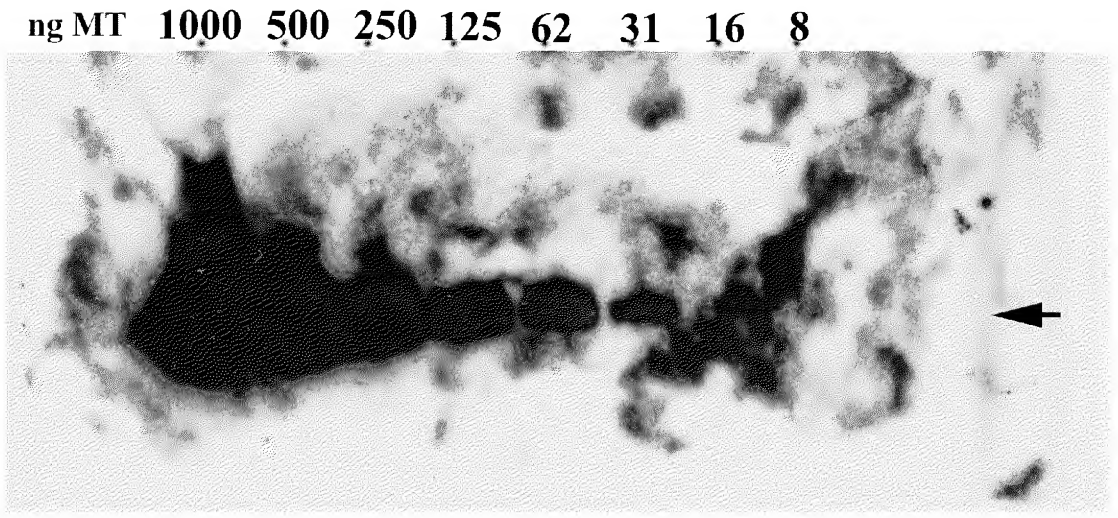

Figure 2. This western blot was run under the same conditions as for Figure 1. The arrow denotes where the MT protein band is present (7kDa). A MT protein band can be seen in all samples, including the 8 ng sample (although this is partly obscured). This demonstrates that our western blotting technique is capable of detecting at least 8 ng MT. The non-specific staining is quite common when performing western blots at this high level of sensitivity.